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Production of extended-spectrum β-lactamases and the potential indirect pathogenic role of *Prevotella* isolates from the cystic fibrosis respiratory microbiota

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Abstract

Extended-spectrum β-lactamase (ESBL) production and the prevalence of the β-lactamase-encoding gene bla_{TEM} were determined in *Prevotella* isolates (n = 50) cultured from the respiratory tract of adults and young people with cystic fibrosis (CF). Time–kill studies were used to investigate the concept of passive antibiotic resistance and to ascertain whether a β-lactamase-positive *Prevotella* isolate can protect a recognised CF pathogen from the action of ceftazidime in vitro. The results indicated that approximately three-quarters (38/50; 76%) of *Prevotella* isolates produced ESBLs. Isolates positive for ESBL production had higher minimum inhibitory concentrations (MICs) of β-lactam antibiotics compared with isolates negative for production of ESBLs ($P < 0.001$). The bla_{TEM} gene was detected more frequently in CF *Prevotella* isolates from paediatric patients compared with isolates from adults ($P = 0.002$), with sequence analysis demonstrating that 21/22 (95%) partial bla_{TEM} genes detected were identical to $bla_{TEM-116}$. Furthermore, a β-lactamase-positive *Prevotella* isolate protected *Pseudomonas aeruginosa* from

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the antimicrobial effects of ceftazidime ($P = 0.03$). *Prevotella* isolated from the CF respiratory microbiota produce ESBLs and may influence the pathogenesis of chronic lung infection via indirect methods, including shielding recognised pathogens from the action of ceftazidime.

Keywords

Anaerobes; Cystic fibrosis; β-Lactams; Antibiotic resistance

1. Introduction

Prevotella spp. are dominant obligate anaerobic bacteria belonging to the oral and respiratory microbiota. They have been shown to occur in healthy individuals and in people with chronic pulmonary infection including cystic fibrosis (CF), chronic obstructive pulmonary disease and bronchiectasis [1–8]. This opportunistic pathogen has the potential to produce virulence factors that inhibit the action of antibiotics, facilitate immune evasion and contribute to tissue degradation [9,10]. Nevertheless, the pathogenesis of *Prevotella* infection in chronic polymicrobial lung diseases is not understood.

Pulmonary infection in CF is managed using a range of antimicrobial agents, including β-lactam antibiotics, which target pathogens such as *Pseudomonas aeruginosa* [11]. Although *Prevotella* spp. are not currently targeted by antibiotic treatment of chronic lung infections, we recently detected resistance to penicillin and cephalosporin antibiotics in CF isolates from this genus, with reduced susceptibility associated with β-lactamase production [9]. Extended-spectrum β-lactamases (ESBLs), classed in functional subgroup 2be, including those encoded by a TEM-type β-lactamase gene (bla_{TEM}), are known to confer resistance to such β-lactam antibiotics [12]. ESBL-producing bacteria have also been associated with clinical failure of cephalosporin antibiotics and increased mortality in hospital-acquired infections [13,14]. These data suggest that it may be clinically important to determine whether *Prevotella* spp. specifically produce ESBLs, which is currently unknown. Furthermore, in polymicrobial infections, β-lactamase-producing bacteria may protect β-lactam-susceptible members of the community from antimicrobial agents [15]. Therefore, β-lactamase production by *Prevotella* spp. may be an indirect method by which this genus contributes to disease pathogenesis in CF by enabling recognised pathogens to persist in the presence of β-lactam antibiotics.

The objective of this study was to investigate the production of ESBLs by clinical *Prevotella* isolates ($n = 50$) from the respiratory microbiota of adults and young people with CF and to establish the prevalence of bla_{TEM} among these isolates. We also investigated the concept of passive resistance and hypothesised that a β-lactamase-producing *Prevotella* isolate could protect a *P. aeruginosa* isolate (from CF sputum) from the action of ceftazidime, an antibiotic commonly used in the treatment of CF pulmonary exacerbations.

2. Materials and methods

2.1. Clinical Prevotella isolates

Fifty *Prevotella* isolates from people with CF living in Northern Ireland were included in this study to investigate ESBL production. The CF isolates were cultured from 14 adult (≥18 years) CF patients attending the adult CF clinic, Belfast, UK (sputum, $n = 25$) and 11 paediatric (6–17 years) CF patients attending the paediatric CF clinic, Belfast, UK (sputum, $n = 4$; cough swab, $n = 21$). The isolates originated from single bacterial colonies with different morphotypes from each clinical sample and those identified as *Prevotella* spp. were subsequently used in this study. Isolates were identified via 16S rRNA sequencing (Fig. A1, Appendix). Current prescription of long-term antibiotics (flucloxacillin, azithromycin, tobramycin and colistin) was documented for each CF patient (Table A1, Appendix).

2.2. Extended-spectrum β-lactamase production by Prevotella

Prevotella isolates ($n = 50$) were tested for production of ESBLs under strict anaerobic conditions using the combined disk method according to the manufacturer's instructions. Briefly, each isolate was inoculated onto supplemented Brucella blood agar (SBBA) with disks (Neo-Sensitabs™; Rosco Diagnostica, Taastrup, Denmark) containing the indicator cephalosporins (ceftazidime 30 μg and cefotaxime 30 μg) as well as ceftazidime + clavulanic acid (30 μg + 10 μg) and cefotaxime + clavulanic acid (30 μg + 10 μg). Isolates were classed as ESBL-positive or ESBL-negative according to Clinical and Laboratory Standard Institute (CLSI) guidelines [16] as follows: isolates were identified as ESBL-positive if the diameter of the inhibition zone was increased by ≥5 mm when the tested indicator cephalosporin was combined with clavulanic acid for at least one of the combinations; and isolates were identified as ESBL-negative if the difference between the zone diameters was <5 mm for both combinations. *Klebsiella pneumoniae* ATCC 700603 was used as a quality control strain.

2.3. Susceptibility of Prevotella isolates to β-lactam antibiotics

Amoxicillin, ceftazidime and amoxicillin/clavulanic acid (AMC) were selected for in vitro susceptibility testing as these β-lactams are among the antibiotics recommended for the treatment of CF respiratory infection according to clinical guidelines [17]. Under strict anaerobic conditions, *Prevotella* isolates ($n = 50$) were inoculated onto SBBA and the minimum inhibitory concentrations (MICs) determined by Etest (bioMérieux, Marcy-l'Étoile, France) according to the manufacturer's instructions. *Streptococcus pneumoniae* ATCC 49619 (amoxicillin), *P. aeruginosa* ATCC 27853 (ceftazidime) and *Bacteroides fragilis* ATCC 25285 (AMC) were used as quality control strains. Where anaerobic breakpoints are available, isolates were categorised as susceptible, intermediate-resistant or resistant as defined by CLSI guidelines [18].

2.4. PCR amplification of bla_{TEM} and DNA sequencing

DNA extraction was performed as previously described [19]. An alignment of 109 different $bla_{TEM\text{-}type}$ nucleotide sequences (861 bp, downloaded from BLAST) was used to identify consensus regions for primer annealing. Primers (bla_{TEM}-Forward, 5′-CCG AAG AAC

GTT TTC CAA TG-3′; and *bla*$_{TEM}$-Reverse, 5′-GAA GCT AGA GTA AGT AGT TCG-3′) had 100% coverage with the reference sequences and were purchased from Eurofins MWG Operon (Wolverhampton, UK). The PCR screening assay was performed using My*Taq*™ Red Mix (Bioline, London, UK). The final reaction mixture (50 μL) contained 0.2 μM of each forward and reverse primer and 2 μL of DNA template. *Escherichia coli* NCTC 11560 (*bla*$_{TEM-1}$) was used as a positive control. PCR was performed using an Applied Biosystems® Veriti® Thermal Cycler (Thermo Fisher Scientific, Paisley, UK) with the following parameters: initial denaturation at 94 °C for 5 min; 33 cycles of denaturation at 94 °C for 1 min, annealing at 55 °C for 1 min and extension at 72 °C for 1.5 min; and a final extension at 72 °C for 10 min. Amplicons (424 bp) were analysed as previously described [19]. PCR products positive for *bla*$_{TEM}$ were purified using a QIAquick® PCR Purification Kit (QIAGEN, Manchester, UK) in accordance with the manufacturer's instructions. Both strands of DNA were sequenced by Eurofins MWG Operon using the forward and reverse primers, and a consensus DNA sequence was determined with CLUSTALW software. The deduced nucleotide sequences were compared with sequences deposited in GenBank.

2.5. Passive resistance

A β-lactamase-positive *Prevotella* isolate (ceftazidime MIC > 256 mg/L) and a β-lactamase-negative *P. aeruginosa* isolate (ceftazidime MIC = 0.5 mg/L) (cultured from CF sputum and identified using 16S rRNA sequencing) were selected to investigate the concept of passive resistance [15].

Time–kill studies were carried out under strict anaerobic conditions according to CLSI guidelines [20]. Assays (*Prevotella* monoculture, *P. aeruginosa* monoculture and co-culture) were performed using basal anaerobic medium containing 1% w/v potassium nitrate (details of this medium are provided in the Appendix) and a cephalosporin antibiotic (ceftazidime; AAH Pharmaceuticals, Belfast, UK) at a predetermined concentration of 64× (32 mg/L) the *P. aeruginosa* MIC; this concentration has also been detected in CF sputum [21]. The initial inoculum of each bacterium was prepared to ca. 5×10^5 CFU/mL. No drug assays were included as controls. Killing activity was assessed at 0, 2, 4, 6, 24 and 48 h and was repeated on three different occasions with colonies enumerated on SBBA (under anaerobic conditions) and on Mueller–Hinton agar (under aerobic conditions) for the *Prevotella* and *P. aeruginosa* isolates, respectively. Drug carry-over was minimised by carrying out serial dilutions (10^{-1} to 10^{-6}) in sterile saline.

A protective effect (antagonism) by *Prevotella* was defined as a ≥2 $\log_{10}$ increase in viable count of *P. aeruginosa* at 48 h compared with that of the *P. aeruginosa* isolate alone [22]. The limit of detection was 2×10^2 CFU/mL. To investigate whether survival of *P. aeruginosa* in the presence of *Prevotella* was secondary to the development of ceftazidime resistance, susceptibility testing (Etest) was performed using individual colonies (n = 10) isolated from the co-culture.

2.6. Statistical analysis

As the combined disk method is not routinely used to analyse ESBL production in *Prevotella* spp., a Spearman's rank correlation coefficient was used to measure the degree of

association between zone diameter in the ESBL detection assay and susceptibility (MICs) to β-lactams. MICs were compared in ESBL-positive versus ESBL-negative isolates using the Mann–Whitney test. The prevalence of ESBL-producing isolates cultured from CF patients currently prescribed long-term antibiotics (flucloxacillin, azithromycin, tobramycin and colistin) compared with CF patients not prescribed these antibiotics was also determined using a χ^2 test with Yates continuity correction or Fisher's exact test. To investigate whether there was an association between the number of long-term antibiotics prescribed (0, 1, 2 or ≥3) and detection of ESBL-producing *Prevotella* isolates, Fisher's exact test was used. A χ^2 test with Yates continuity correction was also utilised to determine whether there was evidence of an association between ESBL production or detection of bla_{TEM} and *Prevotella* group (CF adult versus CF paediatric patients). The total viable count ($\log_{10}$ CFU/mL) of *P. aeruginosa* in the presence of ceftazidime ± *Prevotella* was compared using a paired samples *t*-test. All statistical analyses were carried out with IBM SPSS Statistics for Windows v.21.0 (IBM Corp., Armonk, NY). A two-tailed *P*-value of <0.05 was considered statistically significant.

3. Results

3.1. Extended-spectrum β-lactamase production

ESBL production was common among *Prevotella* isolates from people with CF and was associated with high MICs of β-lactam antibiotics. Fig. A1 summaries ESBL production among the different clinical *Prevotella* spp. isolated from adult and paediatric CF patients (Appendix). The size of the measured zones of inhibition, obtained with the combined disk assay compared with MICs of the β-lactam antibiotics in ESBL-positive and ESBL-negative *Prevotella* isolates are summarised in the Appendix (Fig. A2). There was a strong inverse relationship in all analyses between zone diameter of the indicator cephalosporin antibiotics and susceptibility, with higher MICs of amoxicillin, ceftazidime and AMC associated with smaller zones of inhibition ($P < 0.001$) (Fig. A2, Appendix). Several ESBL-positive *Prevotella* isolates were identified as having apparently weak enzyme activity; these isolates had low MICs with correspondingly large zones of inhibition and clustered with the ESBL-negative isolates (Fig. A2, Appendix). Similar weak in vitro ESBL activities have been identified in other bacterial genera and correlated with clinical failure in patients [13].

Approximately three-quarters of isolates tested (38/50; 76%) were classed as ESBL-positive. MIC results are summarised in Table 1. Resistance to the three β-lactam antibiotics was higher in ESBL-positive isolates compared with ESBL-negative isolates as demonstrated by higher MIC_{50} and MIC_{90} values among the former group. Furthermore, 23 (61%) of the 38 ESBL-producing isolates were also categorised as resistant ($n = 15$) or intermediate-resistant ($n = 8$) to AMC. In contrast, all ESBL-negative isolates ($n = 12$) were susceptible to AMC. Moreover, the ESBL-positive isolates had significantly higher MICs of amoxicillin, ceftazidime and AMC compared with ESBL-negative isolates ($P < 0.001$, Mann–Whitney test) (Fig. 1). The Appendix also details ESBL production by multiple *Prevotella* isolates cultured from an individual clinical sample provided by some people with CF ($n = 18$) (Table A2); the majority of people either harboured ESBL-positive isolates only (10/18; 56%) or both ESBL-positive and ESBL-negative isolates (7/18; 39%).

3.2. Long-term prescription of antibiotics

Long-term antibiotic pressure may select for antibiotic resistance [11], and in our CF patient cohort four oral or inhaled antibiotics were prescribed chronically (oral: azithromycin and flucloxacillin; inhaled: tobramycin and colistin) (Table A1, Appendix). Therefore, we determined whether there was an association between detection of ESBL-producing isolates in patients currently prescribed these long-term antibiotic treatments compared with patients not prescribed the antibiotics. A similar percentage of ESBL-producing isolates was recovered from both groups (Fig. 2). In addition, we analysed whether there was a correlation between detection of ESBL-producing isolates and current chronic prescription of 0 (ESBL-positive, 86%), 1 (ESBL-positive, 83%), 2 (ESBL-positive, 67%) or ≥3 (ESBL-positive, 63%) antibiotics (Table A1, Appendix). As the number of long-term antibiotic treatments prescribed increased, the prevalence of ESBL-producing *Prevotella* isolates decreased; however, no statistical difference was detected ($P = 0.180$, Fisher's exact test).

3.3. The bla_{TEM} gene

Twenty-two (44%) of the fifty clinical *Prevotella* isolates and *E. coli* NCTC 11560 were positive for bla_{TEM} using the PCR screening assay. Sequence analysis revealed that of the 22 bla_{TEM} partial genes (ca. 400 bp) from *Prevotella*, 21 (95%) sequences had 100% identity with $bla_{TEM\text{-}116}$. As expected, the partial nucleotide sequence from *E. coli* NCTC 11560 had 100% identify with $bla_{TEM\text{-}1}$. Furthermore, 18/22 (82%) bla_{TEM}-positive *Prevotella* isolates were also positive for ESBL production using the combined disk method.

3.4. Prevotella isolates from paediatric or adult cystic fibrosis patients

ESBL production and presence of bla_{TEM} were compared between those isolates cultured from adult versus paediatric CF patients. There was no association between CF group (adult versus paediatric) and ESBL production ($P = 0.74$, $\chi^2 = 0.110$) (Fig. 3), with 20/25 isolates (80%) from adult patients and 18/25 isolates (72%) from paediatric patients positive for ESBL production. However, a significant association was identified between CF group (adult versus paediatric) and the presence of bla_{TEM}; 5 (20%) of 25 isolates from adult patients were positive for the gene compared with 17/25 (68%) isolates from paediatric patients ($P = 0.002$, $\chi^2 = 9.821$) (Fig. 3).

3.5. Passive resistance

As polymicrobial lung infection is frequent in CF, we tested whether *Prevotella* may shield *P. aeruginosa* from antibiotics. At 48 h (Fig. 4), the difference in *P. aeruginosa* viable count between the co-culture and monoculture was >2 $\log_{10}$ CFU/mL [mean co-culture viable count, 1.63×10^6 CFU/mL; mono-culture, $<2 \times 10^2$ CFU/mL (limit of detection)], indicating a protective effect by the β-lactamase-positive *Prevotella* isolate. Moreover, the difference in total viable count of *P. aeruginosa* at this time point was statistically greater when cultured with compared to without the *Prevotella* isolate ($P = 0.03$, paired samples *t*-test) (Fig. 4). We also found that survival of *P. aeruginosa* in the presence of *Prevotella* was not secondary to development of ceftazidime resistance [mean MIC = 0.22 mg/L ($n = 10$)].

4. Discussion

The *Prevotella* genus is commonly identified as belonging to the respiratory microbiota in people with chronic lung disease using molecular methods [1,2,8], and complex culture methods also detect *Prevotella* spp. in approximately one-third of CF pulmonary samples [4]. Despite evidence demonstrating the clinical importance of *Prevotella* at other body sites [23], we do not currently understand the role of this obligate anaerobe in infection and inflammation in chronic lung diseases. This is the first study to show that production of ESBLs is common among CF *Prevotella* isolates, which was associated with reduced susceptibility to β-lactam antibiotics. Future studies determining *Prevotella* susceptibility should consider production of this enzyme as a potentially important mechanism of resistance. We also present novel work investigating how β-lactamase-producing isolates from this genus may contribute to the pathogenesis of chronic lung infection and found that a β-lactamase-positive *Prevotella* isolate could shield *P. aeruginosa* from the antimicrobial action of ceftazidime.

Using the nitrocefin test [9], we previously identified that a high number of clinical *Prevotella* isolates from CF and non-CF sources produced β-lactamases (91/153; 59%). In this earlier study, we also found that β-lactamase production was correlated with reduced susceptibility to ceftazidime [9]. ESBLs, which belong to functional subgroup 2be [12], are one type of β-lactamase that have been associated with this spectrum of activity. ESBLs have also been associated with treatment failure with cephalosporin antibiotics and increased mortality in nosocomial infections [13,14]. However, in vitro production of ESBLs by *Prevotella* spp. has not been extensively investigated, with a single bacteraemia isolate identified as ESBL-positive in a case study reported by Mory et al. [24]. The combined disk method is used by clinical laboratories to confirm production of ESBLs [12]. In the current study, 38/50 (76%) *Prevotella* isolates tested were ESBL-positive using this method, and production of the enzyme was associated with significantly higher MICs of amoxicillin and ceftazidime. Although ESBLs are characteristically inhibited by clavulanic acid [12], the ESBL-positive isolates in this study also demonstrated reduced susceptibility to AMC. Mechanisms that may confer resistance to AMC include limiting access of the antibiotic to the target site via reduced permeability and expression of efflux pumps [19]. We previously demonstrated that expression of efflux pumps was not associated with AMC resistance amongst *Prevotella* spp. [19]; however, the role of outer membrane porins and reduced susceptibility to this antibiotic could be investigated.

Chronic antibiotic use has been correlated with an increased prevalence of multidrug-resistant bacteria in CF [11]. Long-term antibiotic treatments were commonly prescribed in the CF patient cohort included in this study and this fact prompted us to investigate the effect of such chronic antibiotic use on the prevalence of ESBL-positive *Prevotella* isolates [11]. However, no difference was detected between patients currently prescribed or not prescribed chronic antibiotic treatments and detection of ESBL-positive isolates. Intermittent episodes of acute pulmonary exacerbations are treated with additional antibiotics in CF. The effect of such short-term antibiotic use on ESBL-production among *Prevotella* isolates could form the basis of future studies.

Multiple *bla*$_{\text{TEM-type}}$ genes have been described, which belong to functional subgroups 2b, 2be, 2br and 2ber β-lactamases; these subgroups reflect the enzymes substrate spectrum [12]. For example, *bla*$_{\text{TEM-1}}$ and *bla*$_{\text{TEM-2}}$ encode β-lactamases that belong to subgroup 2b and confer resistance to penicillins and some cephalosporin antibiotics [12]. Amino acid substitutions within these two β-lactamases subsequently led to the description of ESBLs, in subgroup 2be, with a broadened spectrum of activity that includes the oxyimino-β-lactams such as ceftazidime [12]. Previously, *bla*$_{\text{TEM}}$ was found in 1/4 (25%) *Prevotella* endodontic strains tested [25]. In the current study, we detected *bla*$_{\text{TEM}}$ in 22/50 (44%) isolates using PCR-based detection. Sequence analysis revealed that 21/22 (95%) partial *bla*$_{\text{TEM}}$ nucleotide sequences had 100% identity with *bla*$_{\text{TEM-116}}$. This gene has been detected previously in *E. coli* and *K. pneumoniae* isolates and was associated with high MICs against a range of β-lactam antibiotics including ceftazidime [26]. We also showed that 18/38 (47%) ESBL-positive isolates harboured *bla*$_{\text{TEM}}$. Although these data provide some evidence to suggest that *bla*$_{\text{TEM}}$, associated with *Prevotella* isolates, encodes an ESBL, further work is required to confirm this hypothesis. To characterise the role of this gene in antibiotic resistance in *Prevotella*, β-lactam susceptibility could be determined in a *bla*$_{\text{TEM}}$ knock-out strain. Furthermore, the results of this study potentially suggest that *bla*$_{\text{TEM}}$ detected amongst CF *Prevotella* isolates is geographically clustered: all isolates were cultured from people living in Northern Ireland and we observed the majority of isolates to harbour the same partial *bla*$_{\text{TEM-type}}$ gene.

We cannot discount that other isolates included in this study did not harbour *bla*$_{\text{TEM}}$ owing to our primers not recognising the target gene as a result of mutations within the target sequence. However, it is also possible that *Prevotella* isolates possess a different β-lactamase-encoding gene, e.g. those that encode SHV or CTX-M ESBLs. Furthermore, other enzymes that have been correlated with reduced susceptibility to β-lactam antibiotics in *Prevotella* spp. are CfxA-type β-lactamases, in functional subgroup 2e [27]. Previous work indicated that this β-lactamase has penicillinase and cephalosporinase properties with low MICs of third- and fourth-generation cephalosporins apparent, including cefotaxime and ceftazidime [27]. Giraud-Morin et al. also demonstrated that all *Prevotella* isolates positive for a *cfxA*-type gene (n = 62) were susceptible to ceftazidime [28]. In contrast, in our earlier study we identified an association between the presence of the *cfxA*-type gene and both amoxicillin and ceftazidime resistance amongst *Prevotella* isolates from various sources [19]. The reduced ceftazidime susceptibility observed may be due to mutations within the amino acid sequence of the CfxA-type β-lactamase, which are spectrum extending, or be the consequence of a different resistance mechanism [19,28]. If the former mechanism exists, then it is unclear whether production of such a CfxA-type β-lactamase by some *Prevotella* spp. would result in an ESBL phenotype that is detected by the combined disk method.

Moreover, *Prevotella* isolates may possess more than one gene encoding a β-lactamase. Therefore, a systematic evaluation of β-lactamases in *Prevotella* spp. should now be undertaken, e.g. using analytical isoelectric focusing.

The *Prevotella* isolates from paediatric CF patients were more likely to harbour *bla*$_{\text{TEM}}$ compared with isolates from adult CF patients. This may be due to the fact that *bla*$_{\text{TEM}}$ is usually plasmid-mediated and it is possible that in older CF patients, where the antibiotic

treatment burden is greater compared with younger patients, resistance mechanisms are more likely to be chromosomally encoded. To investigate this theory, a longitudinal study is needed to assess changes in the location and expression of ESBL-encoding genes in isolates during disease progression.

Given that *Prevotella* spp. are detected as part of a polymicrobial infection in the respiratory tract, there is the potential that β-lactamase-producing *Prevotella* facilitate the persistence of susceptible isolates, from the same or different genus, in lethal concentrations of β-lactam antibiotics [15]. A recent study found that β-lactamase-producing *Bacteroides* spp. were able to shield a β-lactamase-negative *E. coli* isolate from the activity of ceftriaxone [29]. Likewise, we have shown that a β-lactamase-positive *Prevotella* isolate protected a susceptible *P. aeruginosa* isolate from the action of ceftazidime. Enabling this recognised pathogen to persist in the presence of ceftazidime may therefore be an indirect method by which *Prevotella* contribute to chronic lung infection, and studies focusing on passive antibiotic resistance are necessitated to confirm this finding with a larger panel of isolates and genera. A limitation of the in vitro co-culture model is that the CF pulmonary microbiota is complex with a diverse array of bacteria present and the existence of numerous intricate microbial interactions, which may also impact the survival of pathogens in the presence of antibiotics. Moreover, it is unlikely that ceftazidime would be administered alone in the treatment of CF respiratory exacerbations and this is not reflected by our current in vitro model. Nevertheless, when deciding on appropriate antibiotic treatment for pulmonary infection, clinicians may need to consider production of β-lactamases by all bacteria belonging to the microbiota, which subsequently protect themselves and other members of the community from antimicrobial agents.

5. Conclusions

In summary, we have shown that *Prevotella* isolated from CF airways infection produce ESBLs, which potentially contribute to reduced susceptibility to β-lactam antibiotics. Genotypic analysis revealed that >40% of *Prevotella* isolates harboured bla_{TEM}, a clinically important β-lactamase-encoding gene in other bacterial genera. These data suggest that future studies investigating *Prevotella* susceptibility should examine the production of ESBLs by this genus. This study also supports the hypothesis that β-lactamase-producing *Prevotella* spp. shield recognised CF pathogens from treatment with ceftazidime, potentially contributing to the persistence of lung infection.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Highlights

- We describe ESBL production by *Prevotella* from the cystic fibrosis respiratory microbiota.
- ESBL production was common among *Prevotella* isolates.
- Higher MICs of β-lactam antibiotics were detected in ESBL-producing isolates.
- The bla_{TEM} gene was detected in >40% of *Prevotella* isolates.
- A β-lactamase-positive *Prevotella* isolate shielded *Pseudomonas aeruginosa* from ceftazidime.

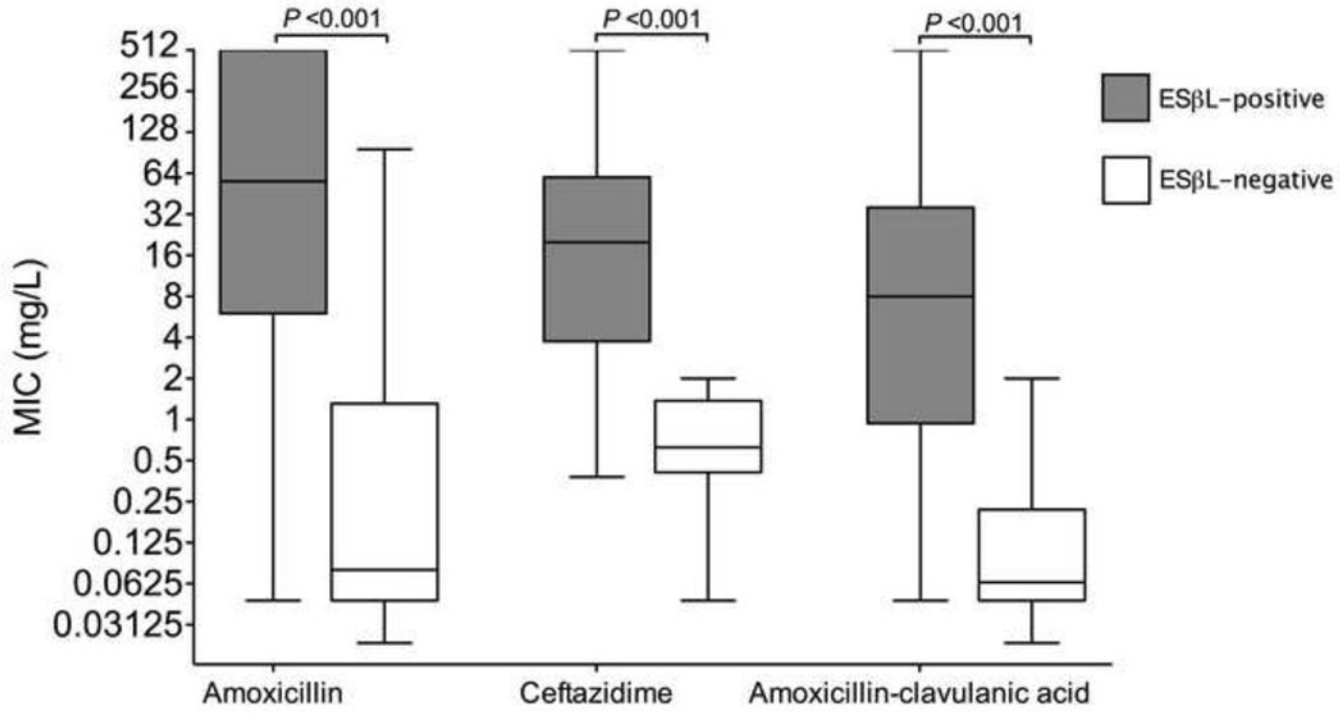

Fig. 1.

Comparison of susceptibility (MICs) between ESBL-positive (n = 38) and ESBL-negative (n = 12) cystic fibrosis *Prevotella* isolates. In the box and whisker plot, the top and bottom boundaries of each box indicate the 75th and 25th quartile values, respectively, with the line inside the box representing the median (50th quartile). End of whiskers indicate the range. Any isolates recorded as having an MIC greater than the maximum (>256 mg/L) concentration on the Etest strip are shown as double the maximum concentration. MIC, minimum inhibitory concentration; ESBL, extended-spectrum β-lactamase.

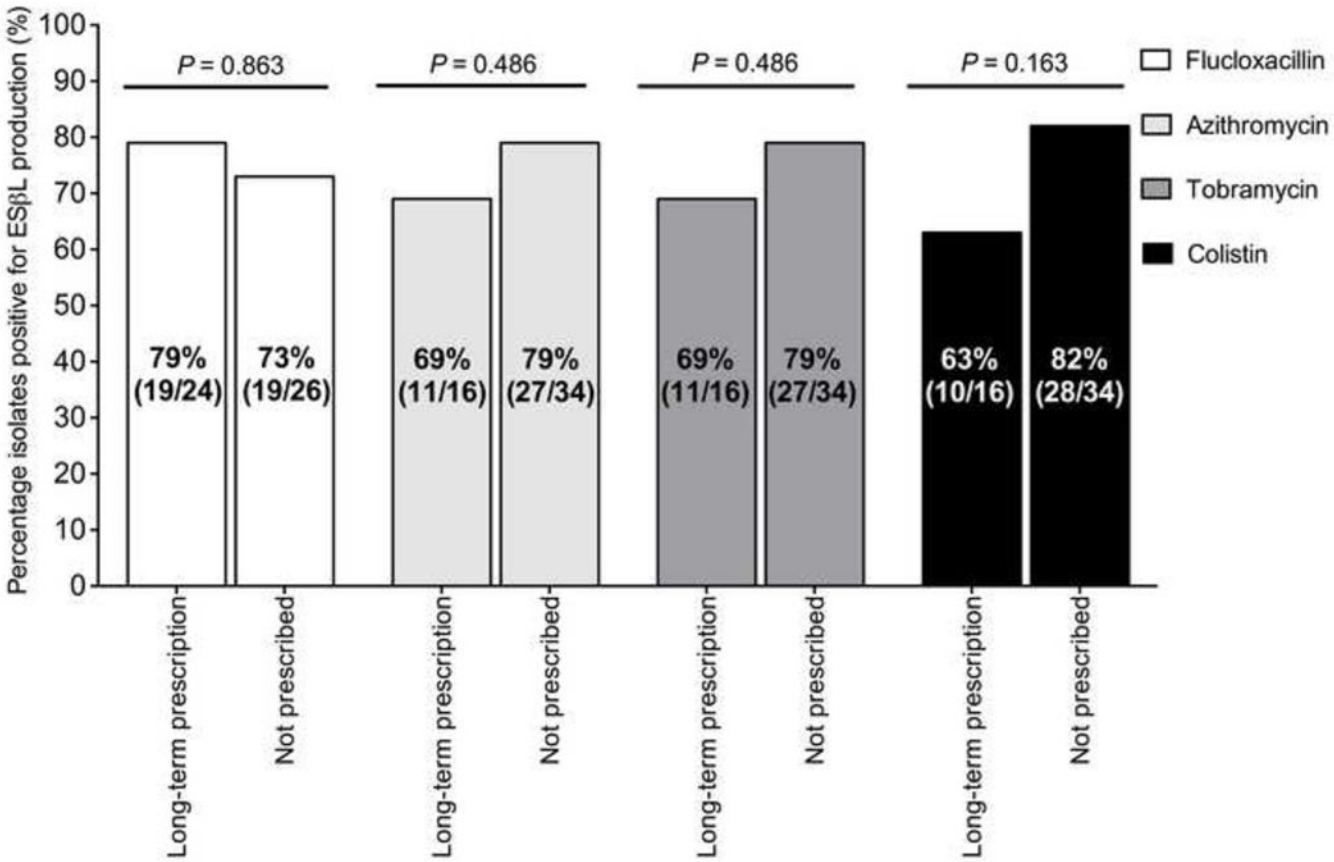

Fig. 2.

Prevalence of ESBL-producing *Prevotella* isolates from CF patients prescribed long-term antibiotics (flucloxacillin, azithromycin, tobramycin and colistin) compared with CF patients not prescribed chronic antibiotic treatments. ESBL, extended-spectrum β-lactamase; CF, cystic fibrosis.

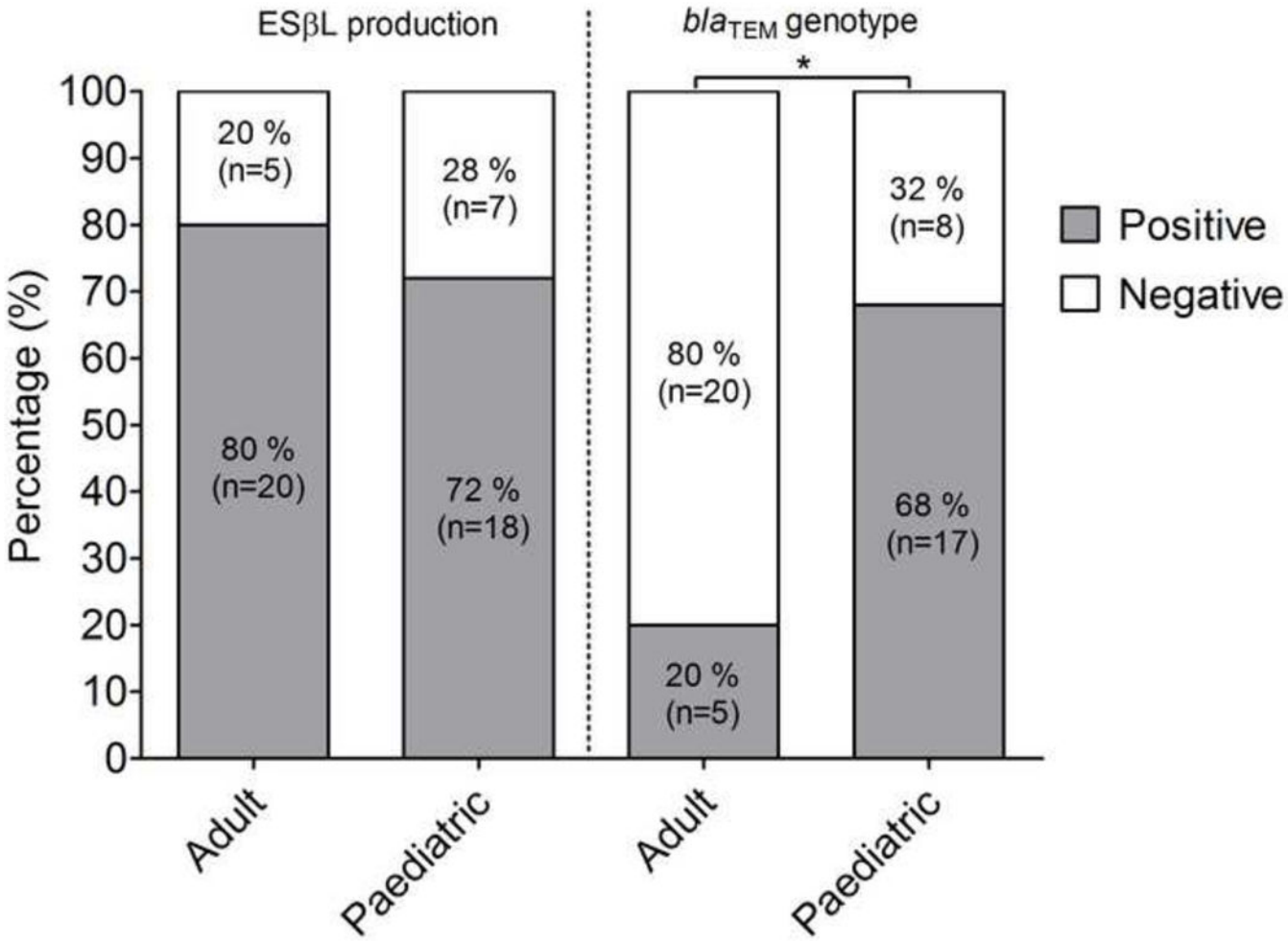

Fig. 3.

Comparison of ESBL production and detection of *bla*$_{TEM}$ between CF adult (n = 25) and CF paediatric (n = 25) isolates. *P < 0.05. ESBL, extended-spectrum β-lactamase; CF, cystic fibrosis.

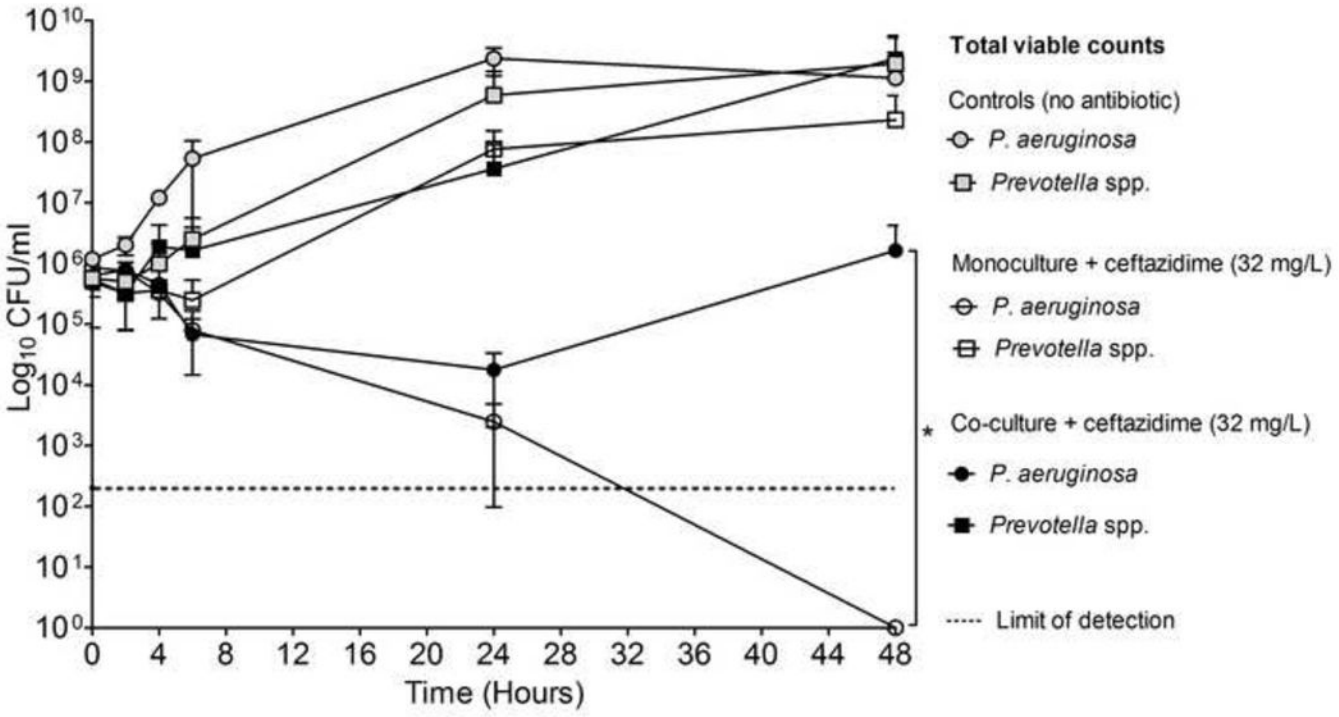

Fig. 4.

Time–kill studies (mean ± standard deviation, $n = 3$) using ceftazidime at 64× the *Pseudomonas aeruginosa* minimum inhibitory concentration (MIC) (32 mg/L). A protective effect (antagonism) by *Prevotella* was defined as a ≥2 $\log_{10}$ increase in viable count of *P. aeruginosa* at 48 h compared with that of the *P. aeruginosa* isolate alone. *$P < 0.05$.

Table 1

In vitro antimicrobial susceptibility of extended-spectrum β-lactamase (ESBL)-positive and ESBL-negative clinical *Prevotella* isolates.

ESBL production	MIC (mg/L)			% of isolates with indicated susceptibility		
	Range	MIC_{50}	MIC_{90}	S	I	R
ESBL-positive (*n* = 38)						
Amoxicillin	0.047 to >256	48	>256	N/A	N/A	N/A
Ceftazidime	0.38 to >256	16	128	N/A	N/A	N/A
AMC	0.047 to >256	8	48	39	21	39
ESBL-negative (*n* = 12)						
Amoxicillin	0.023–96	0.064	2	N/A	N/A	N/A
Ceftazidime	0.047–2	0.5	1.5	N/A	N/A	N/A
AMC	0.047–2	0.064	1	100	0	0

MIC, minimum inhibitory concentration; MIC50/90, MICs that inhibit 50% and 90% of the isolates, respectively; S, susceptible; I, intermediate-resistant; R, resistant; AMC, amoxicillin/clavulanic acid; N/A, no anaerobic breakpoints approved by the Clinical and Laboratory Standards Institute (CLSI).